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GLYCEROL DOES NOT REDUCE NEURONAL DAMAGE IN EXPERIMENTAL *STREPTOCOCCUS PNEUMONIAE* MENINGITIS IN RABBITS

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ABSTRACT

Schmidt H, Stuert K, Chen V, Stringaris AK, Brück W, Nau R. Glycerol does not reduce neuronal damage in experimental *Streptococcus pneumoniae* meningitis. Inflammopharmacology. 1998;6:19–26.

To study the effect of high-dose glycerol therapy on inflammation and neuronal destruction in a model of experimental pneumococcal meningitis, 14 New Zealand White rabbits were infected intracisternally with *Streptococcus pneumoniae* type 3. Sixteen hours after infection, 7 animals received intravenous glycerol therapy (1 g kg⁻¹ bolus and 0.5 g kg⁻¹ h⁻¹ maintenance dose) and 7 animals served as untreated controls.

After 8 h of therapy, the glycerol CSF:serum ratio exceeded the previously observed values in rabbits with an intact blood–CSF barrier (0.72 ± 0.25 vs. 0.35), i.e. glycerol crossed the blood–CSF barrier more readily in animals altered by meningitis than in healthy animals. In contrast, the brain tissue:serum ratio of glycerol (grey matter 0.33 ± 0.29 , white matter 0.30 ± 0.31) was substantially lower than the CSF:serum ratio ($p = 0.03$ and $p = 0.047$). There was no significant effect of glycerol on intracranial pressure, brain water content and neuron-specific enolase release into the CSF. Glycerol significantly increased the density of neuronal apoptosis in the dentate gyrus of the hippocampal formation. Therefore, glycerol does not appear to be beneficial in experimental pneumococcal meningitis.

Keywords: apoptosis, glycerol, meningitis, neuron-specific enolase, *Streptococcus pneumoniae*

INTRODUCTION

For more than 30 years, hyperosmolar glycerol solution has been applied to treat cerebral oedema and intracranial pressure in cerebral ischaemia [1]. Several studies reported positive effects on survival and neurological sequelae in patients with cerebral ischaemia [2–4]. Not only ischaemia but also inflammatory cerebral diseases induce cerebral oedema and increase intracranial pressure complicating the course of the disease. In addition to the dehydrating effect on brain tissue, a direct cerebroprotective action has been ascribed to glycerol. The rapid distribution of glycerol within the brain [5] may cause a decreased cellular loss of free fatty acids and inorganic phosphate in damaged areas and a rise of the rate of glucose and oxygen consumption [6]. Glycerol preserves the cerebral ultrastructure in experimental ischaemia after middle cerebral artery occlusion in the squirrel [7]. It has antioxidative properties and may activate the

serum prostaglandin PGI_2 [8] which in turn induces an increase of cerebral blood flow by vasodilation. With moderate hyperosmolarity, in healthy rats glycerol causes increased taurine, aspartic acid, alanine, leucine and lysine levels in brain tissue [9] and may thereby act as a cerebroprotective agent. Kilpi et al. [10] treated children with bacterial meningitis with an oral glycerol regimen ($4.5 \text{ g kg}^{-1} \text{ d}^{-1} \text{ po}$) or dexamethasone ($1.5 \text{ mg kg}^{-1} \text{ d}^{-1} \text{ iv}$) or both as an adjunct to antibiotic treatment. The end points of this study were persisting neurological abnormalities and impairment of hearing. Glycerol-treated children, with or without additional dexamethasone therapy, had significantly fewer neurological abnormalities and hearing impairments than those receiving only antibiotic treatment or adjunctive treatment with dexamethasone.

This study prompted us to evaluate the role of glycerol as an adjunctive agent in *Streptococcus pneumoniae* meningitis with respect to parameters of inflammation and neuronal destruction. We also studied the entry of glycerol into CSF and brain tissue in the presence of meningeal inflammation.

MATERIALS AND METHODS

Test organism

A *Streptococcus pneumoniae* type 3 strain originally isolated from an adult with meningitis (gift of M.G. Täuber MD, Division of Infectious Diseases, University of California, San Francisco) was used as an inoculum. Its ability to cause meningeal inflammation has been characterized previously [11].

Drugs

Glycerol was infused as a 10% solution in a NaCl (0.45%)/Glucose (0.275%) solution (Glycero steril 10%, Fresenius, Bad Homburg, Germany).

Rabbit model

After induction of anaesthesia with intramuscular ketamine (Ketavet, Parke-Davis, Berlin, Germany; 25 mg/kg) and xylazine (Rompun, Bayer, Leverkusen, Germany; 5 mg/kg), New Zealand White rabbits (approximately 2.5 kg) were anaesthetized by intravenous urethane (Sigma Chemicals, Taufkirchen, Germany) for the entire experiment (24 h). Blood was drawn from the contralateral ear artery. A lumbar puncture needle ($22\text{G} \times 3.5 \text{ inches}$; Spinocan, Braun, Melsungen, Germany) was used as an intracisternal dwelling catheter and was connected to a pressure transducer (Gould Statham R23 ID) for measurement of intracerebral pressure (ICP). Data on ICP was stored by a computerized intensive care monitor (Servomed, SMC 108 monitoring device, Hellige, Freiburg, Germany).

Meningitis was induced by intracisternal injection of 10^6 CFU *Streptococcus pneumoniae* type 3. Until the start of glycerol treatment, both groups received $5 \text{ ml kg}^{-1} \text{ h}^{-1}$ of 0.9% saline. At 16 h later, 7 rabbits received a bolus injection of 1 g/kg glycerol ($=10 \text{ ml/kg}$) followed by a continuous glycerol infusion of $0.5 \text{ g kg}^{-1} \text{ h}^{-1}$ ($=5 \text{ ml kg}^{-1} \text{ h}^{-1}$). Seven control animals received the same volume of NaCl (0.9%). Blood (3 ml) and CSF (0.3 ml) were drawn before and at 6 h, 12 h, 18 h and 24 h after infection.

Sample processing

After coagulation, blood was centrifuged at $3000g$ for 5 min and the supernatant was immediately frozen at -80°C . CSF white blood cells were counted in a Fuchs–Rosenthal haemocytometer. Pneumococcal CSF titres were determined by plating $10 \mu\text{l}$ of serial ten-fold dilutions on blood agar plates, which were then incubated overnight at 37°C with 5% CO_2 . Bacterial titres at 6, 12, 18 and 24 h served for determination of log-linear regression analysis ($\delta\log\text{CFU ml}^{-1} \text{ h}^{-1}$). The remaining CSF was immediately centrifuged at $3000g$ for 5 min and the supernatants were stored at -80°C .

At 24 h after infection, the rabbits were sacrificed by iv injection of 75 mg thiopental. Thereafter, the skull was opened and the brain was carefully removed and dissected. One half was immediately weighed and dried for 10 d to constant weight in an oven at 120°C . The brain water content was calculated as grams (g) of water per g of dry weight. The frontal part was dissected away from the remaining cerebral hemisphere to prepare grey and white matter for the measurement of glycerol concentrations. The posterior part containing the hippocampus was fixed in paraformaldehyde and embedded in paraffin blocks.

Determination of glycerol concentrations

After weighing, brain tissue was mechanically homogenized in a glass potter. The specimen was cooled by ice water and exposed for 10 min to a Sonifier Cell Disruptor ultrasound device (Branson Sonic Power, Danbury, CT, USA). After a heating period of 30 min in a 55°C water bath, glycerol was measured enzymatically in the homogenized brain tissue, CSF and serum (test kit No. 148270, Boehringer Mannheim, Germany) [12].

Quantification of neuronal damage

NSE concentrations in CSF were measured with an immunoluminometric assay (LIA-mat NSE Prolifigen, Byk-Sangtec, Dietzenbach, Germany). CSF lactate concentrations were quantified enzymatically (Biosen, Dreieich, Germany), CSF protein content by nephelometry.

The density of apoptotic neurons in the gyrus dentatus of the hippocampus after 24 h of meningitis was visualized by *in situ* tiling [13], quantitated by planimetry (Contron Videoplan, Grundig, Nürnberg, Germany) and expressed as the number of labelled neurons per mm² of hippocampal granular cell layer [14].

Statistics

Data were expressed as means \pm standard deviation (SD). Unpaired two-tailed *t*-test was used for comparison; $p < 0.05$ was considered statistically significant.

RESULTS

Glycerol kinetics

Glycerol readily crossed the inflamed blood–CSF barrier. The mean \pm SD CSF concentration was 2.25 ± 1.15 g/L in normal animals compared with a serum concentration of 3.53 ± 0.68 g/L 24 h after infection. This resulted in a CSF:serum ratio of 0.72 ± 0.25 . Concentrations of glycerol in the CSF were significantly higher than in the white matter (2.25 ± 1.15 g/L vs. 0.91 ± 0.67 g/L, $p = 0.032$) and in the grey matter (2.25 ± 1.15 g/L vs. 1.02 ± 0.70 , $p = 0.029$). (Figure 1). The mean \pm STD, the white matter:serum glycerol ratio (0.30 ± 0.31) and the grey matter:serum glycerol ratio (0.33 ± 0.29) were similar to those found by Waterhouse and Coxon [15] in healthy rabbits 7 h after continuous glycerol infusion (mean = 0.42).

Measurement of ICP

The mean ICP decreased in the first 3 h of treatment after initiation of therapy 16 h after infection, in the glycerol-treated group (differences vs. controls not significant; Table 1).

Parameters of CNS inflammation

The course of the CSF leucocyte count, the bacterial density, and concentration of lactate in the CSF were nearly identical in glycerol-treated and control animals (Table 1). The protein concentrations in the CSF as a parameter of blood–CSF barrier damage were not significantly different (Table 1).

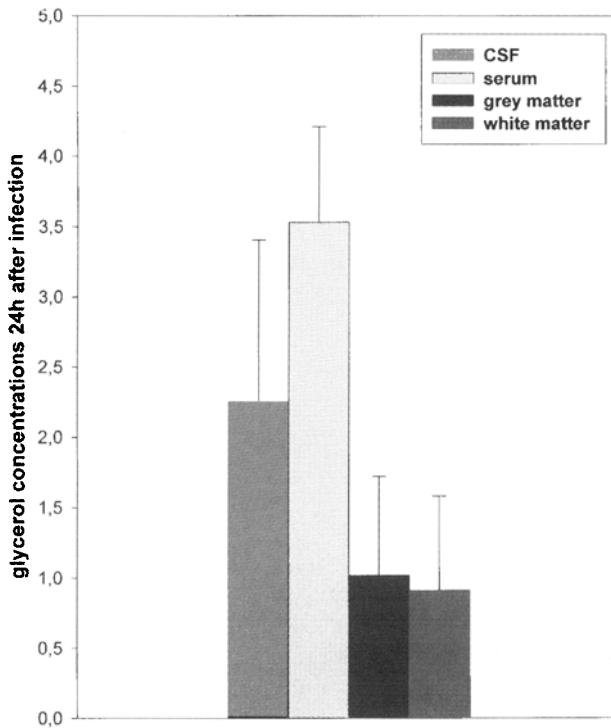


Figure 1. Changes in mean \pm SD of glycerol in CSF, serum and brain tissue 24 h after infection

Determination of brain oedema

The mean brain water content in treated animals was lower than that in control rabbits (3.87 ± 0.65 vs. 4.46 ± 0.38 g_{water} per g_{dry weight}) ($p = 0.07$).

Parameters of neuronal destruction

The concentration of neuron-specific enolase in CSF was not significantly different between glycerol-treated and control animals (mean \pm SD 31.8 ± 25.0 ng/ml vs. 107.8 ± 139.5 ng/ml, $p = 0.17$). The mean density of apoptotic neurons in the dentate gyrus was significantly higher in the glycerol-treated than in the untreated group ($p = 0.0014$; Table 1).

TABLE 1
Effects of glycerol treatment on parameters of meningococcal infection in rabbits

Parameter	Glycerol-treated animals (mean \pm SD)	Control group (mean \pm SD)
CSF leucocyte density ($n/\mu\text{l}$)		
12 h post-infection	1463 \pm 3106	1635 \pm 3515
18 h post-infection	10239 \pm 14380	20751 \pm 29249
24 h post-infection	23800 \pm 34100	28102 \pm 27592
Log CFU/ml		
12 h post-infection	7.9 \pm 1.1	7.0 \pm 0.6
18 h post-infection	9.0 \pm 0.8	8.4 \pm 0.8
24 h post-infection	9.1 \pm 0.6	8.4 \pm 0.6
$\delta\log\text{ CFU ml}^{-1}\text{ h}^{-1}$	0.17 \pm 0.06	0.15 \pm 0.06
ICP (mmHg)		
16 h post-infection	5.5 \pm 5.1	5.0 \pm 8.3
18 h post-infection	4.0 \pm 7.1	2.6 \pm 8.4
21 h post-infection	4.1 \pm 10.6	9.2 \pm 4.8
24 h post-infection	3.3 \pm 10.7	6.3 \pm 8.3
CSF lactate (mmol/L)		
12 h post-infection	3.2 \pm 2.1	7.8 \pm 5.4
18 h post-infection	8.1 \pm 3.6	9.6 \pm 6.0
24 h post-infection	13.5 \pm 9.6	12.9 \pm 6.45
CSF protein content (mg/L)		
6 h post-infection	1613.2 \pm 824.8	1580.8 \pm 1514.5
24 h post-infection	4232.0 \pm 1223.1	3682.7 \pm 2701.5
Density of apoptotic neurons in the dentate gyrus (mm^2)	244.3 \pm 57.1**	130.5 \pm 45.9**

ICP, intracisternal pressure; CSF, cerebrospinal fluid; CFU, colony-forming unit

DISCUSSION

The introduction of antibiotic therapy has decreased the mortality of pneumococcal meningitis from 100% to 20–30%. Nevertheless, in surviving patients, neurological sequelae are frequent. Up to 50% of the survivors suffer from cognitive dysfunction, seizures, hearing impairment or focal neurological abnormalities. These effects are caused partly by the bacteria themselves but also by a sudden activation of the inflammatory cascade induced by bacterial cell wall products, peaking after the initiation of the treatment with cytolytic antibiotics (e.g. β -lactam).

Various efforts have been made to attenuate the inflammatory response of the immune system in early meningitis. The beneficial effect of dexamethasone administration before the start of antibiotic therapy for bacterial meningitis in children of two months or older has been documented in a randomized study [16] and is recommended by several authorities (e.g. the American Academy of Pediatrics [17]). However, dexamethasone treatment of bacterial meningitis increased apoptotic neuronal damage in the dentate gyrus of the hippocampal formation [14]. Moreover, dexamethasone decreases the passage of antibiotics from blood to CSF [18]. Cleavage products of gram-positive bacteria are able to increase intracerebral pressure and brain oedema [19]. Yet, antibiotics acting bactericidally, not by cell wall lysis, such as rifamycins and quinolones delayed the inflammatory response; they did not reduce neuronal damage in experimental pneumococcal meningitis [20, 21].

Studies examining the reduction of brain oedema in meningitis by therapy with osmotic agents are rare. Syrogiannopoulos et al. [22] administered mannitol in a *Haemophilus influenzae* type b meningitis rabbit model and were able to reduce lactate and hypoxanthine concentrations in the CSF of mannitol-treated animals. The Finnish glycerol study [10] claimed a significantly lower percentage of hearing loss and other neurological abnormalities in antibiotic-treated children with bacterial meningitis receiving glycerol alone or plus dexamethasone as adjuvants versus no adjunctive treatment or dexamethasone. The design of this study, however, was inadequate. Thus 30 patients received glycerol alone (group 1) and 34 dexamethasone plus glycerol (group 2). A total of 32 children received dexamethasone alone (group 3) and 26 had no adjunctive treatment (group 4). A comparison of all four groups did not yield significant differences; groups 1 and 2, and groups 3 and 4 were combined, and compared with each other. Furthermore, no adjustment of the α -error was performed to account for repeated testing.

The objective of our study was to evaluate the effect of glycerol on brain oedema and neuronal damage in pneumococcal meningitis. To minimize interfering variables, infected animals were not treated with an antibiotic agent. Glycerol entered the CSF more readily in infected animals than in healthy animals as previously reported by Waterhouse and Coxon [15]. In contrast, glycerol brain tissue:serum ratios in animals suffering from meningitis were almost equal to those in non-meningitic animals [15]. This implies that (a) despite a pronounced disturbance of the blood-CSF barrier the blood-brain barrier appears less affected in early bacterial meningitis, and (b) the long diffusional distances and the bulk flow of the interstitial fluid of the brain tissue towards the subarachnoid space prevents equilibration between brain tissue and CSF.

As in *Haemophilus influenzae* type b meningitis [22], the osmotic treatment resulted in a decrease in mean brain water content and in a slight reduction of intracranial pressure. However, in our study, the differences were not significant. The CSF concentrations of lactate, however, were not affected by the glycerol treatment.

In conclusion, glycerol readily entered the cerebrospinal fluid in experimental meningitis. Its concentrations were substantially lower in brain tissue than in CSF. Glycerol did not reduce parameters of neuronal damage. The design and statistical evaluation of the only study conducted in children claiming a beneficial effect of glycerol as an adjunct in bacterial meningitis is not convincing. For these reasons, at present, there is no indication for routine use of glycerol in bacterial meningitis.

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